

CONFORMATION OF LIQUID *N*-ALKANES

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ABSTRACT The conformations of liquid *n*-alkanes have been studied using neutron scattering techniques to better understand the conformational forces present in membrane lipid interiors. We have studied hydrocarbon chains having lengths comparable to those found for esterified membrane lipid fatty acids, and find that the steric constraints of packing in the liquid state do not change the conformational distributions of hydrocarbon chains from those imposed by the intrachain forces present in the gas phase. It follows that the central region of membranes containing lipids in the disordered state should contain hydrocarbon chain conformations determined primarily by intrachain forces.

INTRODUCTION

Biological membranes containing lipids in the disordered (liquid crystalline) state scatter x-rays and neutrons in a broad band of intensity centered near 4.6 \AA^{-1} (1,2). The scattering from alkanes in the liquid state is very similar in this region (3), and has suggested to some a close similarity of properties with membrane interiors (4). Observations of membrane viscosities support such a contention.

While it would be desirable to study the relative roles of steric interactions, bond rotation potentials and anisotropic origin constraints in natural membranes, it has frequently proved useful to explore simpler systems which permit a more precise characterization. It is, therefore, of some interest to explore the properties of liquid alkanes having chain lengths comparable to those of membrane fatty acids to improve our understanding of the conformations present in membrane lipid interiors and the forces giving rise to those conformations.

In this study we ask whether the steric constraints of packing in the liquid state change the conformational distributions of hydrocarbon chains from those imposed by the intrachain forces present in the gas phase (5). We focus our attention on normal alkane chains between 8 and 16 carbons long, since this is the chain length range for esterified membrane lipid fatty acids in which stable bilayers can be formed (6) and which do not violate the biological requirement for a fluid membrane (7). Saturated chains longer than 16 carbons are not usually found in large amounts in biomembranes, although longer mono- and polyunsaturated chains are common.

Neutron scattering can provide information about the conformation of individual molecules in liquid alkanes through the use of Deuterium labeling. Since neutrons scatter differently from ^1H and ^2H , alkane chains in a chemically homogeneous liquid can be made to contrast with their environment by deuteration. This permits measurement of the radius of gyration of

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the average conformation for an alkane, and ideas about the influence of the environment on conformation can be tested using changes in a measured parameter.

THEORETICAL RESULTS

A single hydrocarbon chain can be characterized by its neutron scattering length density, $\rho = \Sigma bi/V$ where b is the scattering length of the i th atomic nucleus and V is the volume occupied by the molecule. If we use the reciprocal space coordinate $k = 2\pi s = 4\pi \sin \theta/\lambda$ where 2θ is the scattering angle and λ is the neutron wavelength, an interpretation of the scattering of mixtures of deuterated and hydrogenated alkanes can be based on the expression (8–10)

$$I_{\text{TOT}}(k) = S(k)^2 c_H c_D (\rho_H - \rho_D)^2 \quad (1)$$

where $I_{\text{TOT}}(k)$ is the total coherently scattered intensity, $S(k)$ is the Fourier transform of the molecular form factor, c_H and c_D are the fractional composition of natural and perdeuterated hydrocarbons, and ρ_H and ρ_D are the fractional composition length densities. Eq. 1 was originally derived from similar studies on the conformation of polymers in the melt. In that work (8) on the conformation of large H and D polymers in the melt or bulk, it was found to predict accurately the H and D fractional composition dependence of $I_{\text{TOT}}(k)$, without the inclusion of cross terms, indicating negligible interference effects at all fractional H and D compositions. The absence of an interference effect can be ascribed to the multiple conformations and orientations available to the polymers in the melt or bulk.

In the absence of significant intermolecular interference effects, the radius of gyration of a molecule may be measured from the low-angle scattering curve. In general, the radius of gyration R_g is the second moment of the scattering length density, $\rho(r)$ with respect to the center of scattering mass, or $R_g^2 = \int r^2 \rho(r) dv / \int \rho(r) dv$. The radius of gyration of a homogeneous particle depends on the shape of the particle. For example, for ellipsoids of revolution of semi-axes a , a , and wa , (10)

$$R_g = a[(2 + w^2)/5]^{1/2}. \quad (2)$$

R_g can be measured for particles of any shape from the Gaussian region of the curve by the use of a Guinier plot (9) of the $\ln[I(k)]$ as a function of k^2 . The slope, a , of the linear region of this plot is related to R_g by (11)

$$R_g = 3a^{1/2}. \quad (3)$$

In the region of k -space where $k < 1/R_g$, $I(k)$ scattered by a flexible polymer chain is proportional to (12)

$$1 - k^2 R_g^2 / 3. \quad (4)$$

The fact that segments in a chain are connected in a random coil yields a characteristic scattering law. In the absence of excluded volume effects (as is the case for the polymer bulk phase [8]), Debye (13) has shown that $I(k)$ for $k > 1/R_g$ is proportional to

$$(2/x)[x - 1 + \exp(-x)], \quad \text{where } x = k^2 R_g^2.$$

MATERIALS AND METHODS

Neutron Diffraction from Liquid n-Alkanes

Spectrograde *n*-alkanes were purchased from Aldrich Chemical Co. Inc. (Milwaukee, Wis.). Perdeuterated *n*-alkanes were from Merck Isotopes. Samples for the experiments were mixed to a final volume of 300 μ l, loaded into neutron diffraction cells (14) with a Pasteur pipette, and sealed with a sample holder arrangement which prevents the formation of air bubbles by maintaining a seal on the 80- μ l sample through an O-ring.

Low- and high-angle neutron diffraction experiments and data normalization procedures have been described (14).

RESULTS

Neutron Diffraction from Liquid n-Alkanes

Fig. 1 shows the low-angle neutron diffraction from a mixture of 8 mol% D (mol/100 mol) and 92 mol% H dodecane in the liquid phase (45°C), and the corresponding Guinier plot for this data. Model calculations for the all-*trans* conformation of *n*-alkane chains from 8 through 32 carbons indicate that the gaussian approximation (15) used by Guinier should be valid for the range of reciprocal space sampled in these experiments, even if the conformation in the liquid state should approach the limiting case of the all-*trans* model. The results of these calculations are presented in Fig. 3 in the form of a plot of the molecular transform squared obtained from Eq. 2 and the Gaussian function expected from the radius of gyration for the

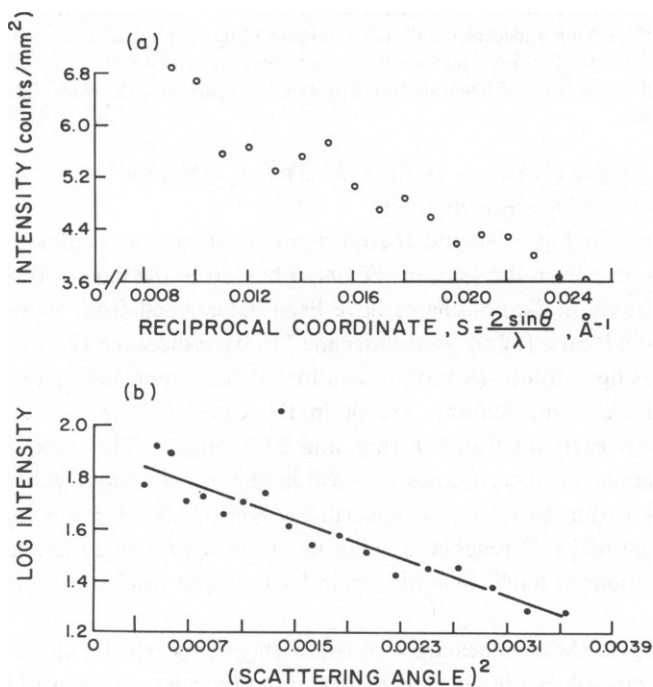


FIGURE 1 Low-angle neutron diffraction from a mixture of 8 mol% D and 92 mol% H dodecane in the liquid phase (45°C) (a), and the corresponding Guinier plot of this data (b).

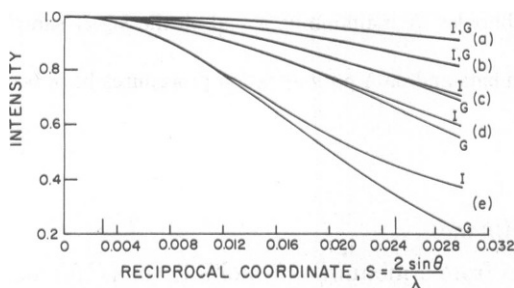


FIGURE 2

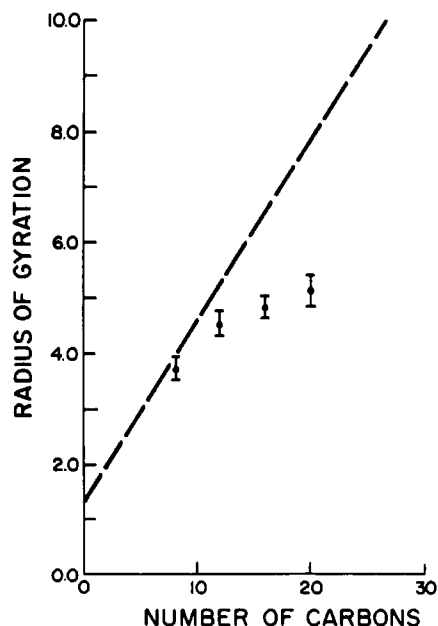


FIGURE 3

FIGURE 2 Match between the square of the molecular transform (I) and the Gaussian approximation (G) for the cylindrical model of an n -alkane in the all-*trans* conformation: (a) n -octane (8:0); (b) n -dodecane (12:0); (c) n -hexadecane (16:0); (d) n -eicosane (20:0); (e) n -dotriacontane (32:0). The marks at $s = 0.003 \text{ \AA}^{-1}$ and $s = 0.025 \text{ \AA}^{-1}$ indicate the experimental range used here.

FIGURE 3 Radii of gyration of liquid n -alkanes at 45°C: comparison with values expected for all-*trans* chains (broken line).

all-*trans* model cylinder (16) $R_g = (r_c^2/2 + h^2/3)^{1/2}$, r_c is the radius and h the half-height of the cylinder containing the all-*trans* chain.

The Guinier plot in Fig. 2 should therefore yield the correct radius of gyration for the n -alkanes studied here from the slope of the linear best fit to the data in this plot. The radii of gyration for the liquid n -alkanes chains have been determined from these Guinier plots for n -octane (8:0), n -dodecane (12:0), n -hexadecane (16:0), n -eicosane (20:0), and n -dotriacontane (32:0), by using a dilute (8 mol%) solution of perdeuterated chains in hydrogenated chain solvents of the same lengths, except in the case of 32:0 (mp 69.7°C), where the determinations were carried out in 8:0, 16:0, and 20:0 solvents. The standard deviations from the linear fit resulted in uncertainties of $\sim 5\%$ in the experimental values for the radii of gyration. Results within this level of uncertainty were found for the radii of gyration in solutions where the mol% D reached as high as 16 mol%; for dodecane and hexadecane at 25°C; and for solutions of mol% D n -alkanes in CCl_4 . These results are summarized in Table I.

As shown in Fig. 4, these values are well below those expected for the all-*trans* models for these chains. It is possible to obtain a rough idea about the average shape of these chains in the liquid phase by assuming that this shape can be approximated by an ellipsoid of revolution, the dimensions of which must be consistent with the radii of gyration and with the molecular volume for each chain (as obtained from the reported values for the mass densities). For an

TABLE I
RADII OF GYRATION OF LIQUID
N-ALKANES

<i>n</i> -Alkanes	Radius of gyration
	\AA
8:0	3.7 ± 0.2
12:0	4.5 ± 0.2
16:0	4.8 ± 0.2
20:0	5.1 ± 0.3
32:0	6.3 ± 0.5

ellipsoid of semiaxes a , a , and wa , $R_g = a[(2 + w^2)/5]^{1/2}$, where a is related to w through the expression for the volume of an ellipsoid: $V = (4/3) a^3 w$, so $a = (3V/4w)^{1/3}$.

Using the expression for a in terms of w it is possible to obtain a fit for the experimental values of the radii of gyration with the best values for w . Two values of w should produce a best fit: one for $0 < w < 1$, corresponding to the best oblate ellipsoid, and another for $w > 1$, corresponding to the best prolate ellipsoid. The results for this analysis are shown in Table II. No values are reported for the 8:0 chains, because no fit was found to be possible for the radius of gyration and molecular volume of this molecule.

The most important result emerging from this analysis is not in the model, but in its apparent constant shape independent of chain length. It is quite naive to assign any particular average shape to a multisegmented molecule in the liquid phase, but it is significant that, if this is attempted, the resulting shape appears to be independent of the number of segments in the molecule. This result, together with the result that the value for the radius of gyration obtained is the same within experimental error in liquid phase as it is in a dilute solution of CCl_4 , argue for an average chain conformation which is rather compact and determined essentially by intramolecular interactions independently of the intermolecular environment if this environment can produce only steric interactions.

Ideas concerning the shape of the diffracting molecules can be tested using the neutron diffraction curve at higher angles. If the complete diffraction curve can be explained on the basis of a model for the conformational distribution of the diffracting molecules, we will have a test for the tentative conclusions obtained from low-angle data. Eq. 5 was obtained by Debye

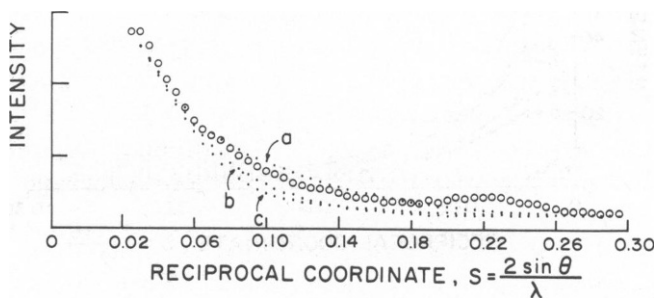


FIGURE 4 Approximations to the average shape of liquid *n*-dodecane. (O) neutron diffraction from an equimolar mixture of H and D *n*-dodecane liquid at 45°C; (a) all-*trans* chain; (b) prolate ellipsoid; (c) oblate ellipsoid.

TABLE II
SIMPLE COMPARATIVE MODELS OF ALKANE
CONFORMATION

<i>n</i> -Alkane	Oblate model		Prolate model	
	<i>w</i>	<i>a</i>	<i>w</i>	<i>a</i>
		(Å)		(Å)
8:0	—	—	—	—
12:0	0.26	7.00	2.86	3.16
16:0	0.28	7.46	2.75	3.49
20:0	0.29	7.90	2.70	3.75
32:0	0.23	9.83	3.06	4.16

(13) by averaging over all forms a flexible polymer can acquire, assuming equal probabilities for all possible configurations around carbon-carbon bonds (random coil). It is possible to attempt a fit of the complete diffraction curve with Eq. 5: if the best fit also requires the use of the value obtained (within experimental error) for the radius of gyration at low angles, then we may conclude that the average conformation of *n*-alkanes in the liquid phase can be approximated by that of a random coil.

Diffraction data through 0.3 \AA^{-1} is shown in Fig. 5 for a 50 mol% mixture of perdeutero *n*-dodecane and *n*-dodecane liquid at 45°C. As shown in Eq. 1, diffraction from a mixture of H and D *n*-alkanes of the same chain lengths results in curves of the same shape, with their actual intensities depending on the product of the mol fractions of D and H in each mixture. The curve obtained for a 50-50 mixture of D and H thus represents the best signal-to-noise which can be obtained for this data, added on the interference function peak [at $(4.6 \text{ \AA})^{-1}$].

Fig. 5 also shows the curves which would be expected for an all-*trans* model and for the ellipsoids of revolution suggested in Table II for this molecule. It can be observed in this figure that the problem of a fit is not a straightforward one, since all of these models do come close to fitting the diffraction curve. But Fig. 6 shows that an even better fit can be obtained with Eq. 5. For *n*-dodecane, the value of the radius of gyration required for this fit is 4.6 ± 0.3 throughout the complete range of fractional H and D compositions. These results confirm the

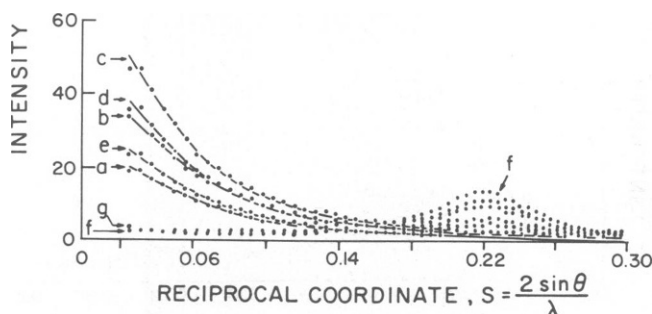


FIGURE 5 Neutron diffraction (●) and curve generated by Eq. 5 (continuous lines) for mixtures of H and D *n*-dodecane liquid at 45°C: (a) 0.13 mol fraction D; (b) 0.25 mol fraction D; (c) 0.50 mol fraction D; (d) 0.75 mol fraction D; (e) 0.87 mol fraction D; (f) 1.00 mol fraction D. The fit to the low-angle data should be compared with that obtained in Fig. 4.

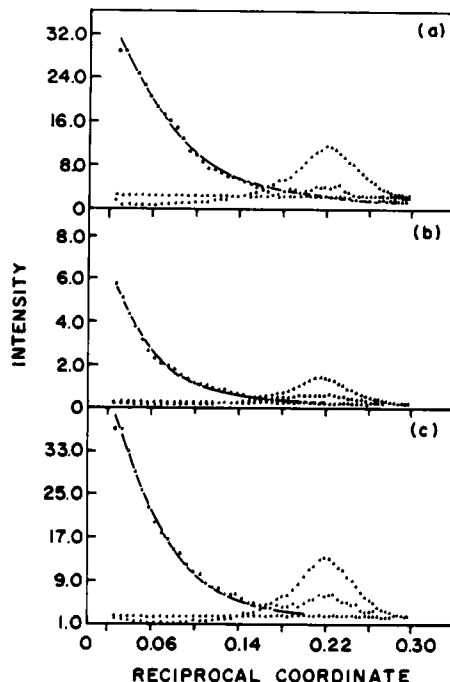


FIGURE 6 Neutron diffraction (●) and curve generated by Eq. 5 (continuous lines) for mixtures of H and D *n*-alkanes corresponding to equimolar mixtures of H and D nuclei in the liquid phase at 45°C: (a) *n*-octane; (b) *n*-hexadecane; (c) perdeutero *n*-octane and *n*-hexadecane. Curves for 0 and 100 mol% D segments are also included for each plot.

validity, within experimental error, of the fractional composition dependence indicated by Eq. 1.

Fig. 6 shows the corresponding fit for the 50-50 curves for 8:OH-8:OD (fit with $R_g = 3.9$ Å), 16:OH-16:OD (fit with $R_g = 5.0$ Å), and 8:OD-16:OH (fit with $R_g = 4.5$ Å). The value found for the 8:OD-16:OH suggests that Eq. 1 is valid even if the two chains do not have the same length, as long as they have the same shape.

DISCUSSION

Conformation of Liquid n-alkanes

The most important conclusion from these experiments is that the conformation of *n*-alkanes in the liquid phase can be considered to be similar to that found for the large hydrocarbon polymers (8-10). This average conformation is the result of an average over all conformations available to these chains at the temperature of the experiments. The number of possible conformations available to a random coil of the type used by Debye (13) in this derivation of Eq. 5 is, by definition, infinite. On the other hand, the number of conformations available to a chain with the constraints shown to be present in the gas phase for the *n*-alkanes, with only *trans*- or *gauche* configurations and ~0.6 kcal difference between the configurational energies of the two structures (5), is very large and should approximate the results for random coils in the chain length range studies reported here. We should expect that an *ab initio* calculation of

the coordinates and weighing factors for each of the conformations expected from the gas phase (5) electron diffraction experiments will yield a function which will match the diffraction curves found in these experiments.

Dettenmaier (17) has studied the conformation of *n*-hexadecane and *n*-hexatriacontane in the melt and in cyclohexane solution. Cyclohexane, like CCl₄, should approximate a θ -solvent, and Dettenmaier found similar radii of gyration for these *n*-alkanes in the melt and in these solvents. The data in this paper were analyzed using calculations of Yoon and Flory (18) based on the rotational isomeric model and found good agreement between these calculations and the low-angle scattering from these molecules.

If no constraints other than those present in the gas phase are responsible for the molecular conformations in liquid hydrocarbon phases, steric effects can be ignored in considering the conformation of chains in the interior of membranes in the fluid state.

A preliminary account of this work was presented at the 21st Annual Meeting of the Biophysical Society (1977) in New Orleans, La. (19).

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